PCSK9 inhibitors: an overview on a new promising lipid-lowering therapy

Denegri, Andrea ; Petrova-Slater, Iveta ; Pasotti, Elena ; Rossi, Maria Grazia ; Pedrazzini, Giovanni Battista ; Moccetti, Tiziano ; Moccetti, Marco

Abstract: Atherosclerosis is characterized by cholesterol deposition in the arterial intima, with subsequent plaque formation and arterial disease. Low-density lipoprotein cholesterol (LDL-C) plays the most important role in the atherogenesis process, which is the substrate of cardiovascular disease and is the leading cause of death worldwide. Several studies show that a strict control of risk factors, particularly the reduction of LDL-C levels, is a cornerstone in primary and secondary prevention of coronary heart disease. Statins are currently the most effective drugs for lowering LDL-C, but the discovery of proprotein convertase subtilisin kexin 9 (PCSK9) has opened up new therapeutic options in lipid management. PCSK9 reduces LDL-receptors' recycling resulting in a decrease of LDL-C receptors on the surface of hepatocytes and an increase of LDL-C levels in plasma. Obviously, inhibition of PCSK9 has been associated with an increase of LDL-C receptors with subsequent lowering of plasma levels of LDL-C. The clinical development of monoclonal antibodies against PCSK9 has been achieved through phase I and II studies, and nowadays there are many ongoing phase III trials with promising preliminary results. The aim of this review is to update the evidence for PCSK9 monoclonal antibodies, such as evolocumab, alirocumab and bococizumab, in LDL-C management and to discuss their therapeutic perspectives based on the most recent clinical studies, with attention to side-effects.

DOI: https://doi.org/10.2459/JCM.0000000000000360

FIC Federazione Italiana di Cardiologia
Italian Federation of Cardiology

PCSK9 inhibitors: an overview on a new promising lipid-lowering therapy

Andrea Denegri, Iveta Petrova-Slater, Elena Pasotti, Maria Grazia Rossi, Giovanni Battista Pedrazzini, Tiziano Moccetti and Marco Moccetti

Atherosclerosis is characterized by cholesterol deposition in the arterial intima, with subsequent plaque formation and arterial disease. Low-density lipoprotein cholesterol (LDL-C) plays the most important role in the atherogenesis process, which is the substrate of cardiovascular disease and is the leading cause of death worldwide. Several studies show that a strict control of risk factors, particularly the reduction of LDL-C levels, is a cornerstone in primary and secondary prevention of coronary heart disease. Statins are currently the most effective drugs for lowering LDL-C, but the discovery of proprotein convertase subtilisin kexin 9 (PCSK9) has opened up new therapeutic options in lipid management. PCSK9 reduces LDL-receptors' recycling resulting in a decrease of LDL-C receptors on the surface of hepatocytes and an increase of LDL-C levels in plasma. Obviously, inhibition of PCSK9 has been associated with an increase of LDL-C receptors with subsequent lowering of plasma levels of LDL-C. The clinical development of monoclonal antibodies against PCSK9 has been achieved through phase I and II studies, and nowadays there are many ongoing phase III trials with promising preliminary results. The aim of this review is to update the evidence for PCSK9 monoclonal antibodies, such as evolocumab, alirocumab and bococizumab, in LDL-C management and to discuss their therapeutic perspectives based on the most recent clinical studies, with attention to side-effects.

J Cardiovasc Med 2016, **17**:237–244

Keywords: alirocumab, evolocumab, familial hypercholesterolemia, hypercholesterolemia, PCSK9 inhibitors

Cardiocentro Ticino, Lugano, Switzerland

Correspondence to Dr Andrea Denegri, MD, Cardiocentro Ticino, Via Tesserete 48, 6900 Lugano, Switzerland
Tel: +393286574387; e-mail: denegriandrea@msn.com/andrea.denegri@usz.ch

Received 11 August 2015 **Revised** 7 December 2015
Accepted 25 December 2015

Introduction

Cardiovascular disease (CVD) is the primary cause of death worldwide, mainly because of the aging of the population.[1] However, improved control of cardiovascular risk factors has led, in the last two decades of the 20th Century, to a consistent reduction in deaths attributable to coronary heart disease.[2] Low-density lipoprotein cholesterol (LDL-C)-lowering therapies have played a primary role in the reduction of cardiovascular risk and still remain a mainstay in coronary heart disease (CHD) secondary prevention. Large randomized clinical trials show a linear relationship between LDL-C reduction and a decrease in CVD and the new AHA/ACC guidelines adopt more and more stringent LDL-C targets.[3] Statins are currently the most effective drugs in reducing LDL-C levels and except for the ezetimibe, no lipid-modifying therapy added to statins has been demonstrated to provide a clinical benefit.[4] Moreover an increase from a high dose to a very high dose of statin leads at best to a further 2% reduction in clinical events, but with the negative consequences of increased side-effects and decreased compliance.[5] The discovery of proprotein convertase subtilisin kexin 9 (PCSK9) is a new and promising therapeutic option in the management of LDL-C levels.[6] The aim of this review is to update the evidence regarding PCSK9 antibodies' treatment in lowering LDL-C levels and discusses its therapeutic prospectives based on the most recent clinical trials.

Features of proprotein convertase subtilisin kexin 9

PCSK9 is a protein secreted mostly from the liver, encoded by the *PCSK9* gene. In 2003, Seidah *et al.*[7] identified the ninth member of the proprotein convertase family (PCSK9) and in the same year another group showed a possible involvement of PCSK9 in regulating cholesterol metabolism.[8] Berberine, a quaternary ammonium salt of protoberberine, is a substance found in plants such as of the genus *Berberis* (hence the name), usually in the roots, rhizomes, stems and bark, characterized by an upregulating activity on low-density lipoprotein receptor (LDLR).[9] PCSK9 binds the epidermal growth factor-like repeat A (EGF-A) domain of the LDLR positioned onto the surface of the hepatocyte, and degrades it intracellularly.[10,11] Reduced LDLR levels result in decreased metabolism of LDL-C, which could lead to hypercholesterolemia. Variants of PCSK9 can reduce or increase circulating cholesterol. LDLR present on the surface of liver cells binds the circulating LDL-C, removes it from the blood and takes it inside the cells. When PCSK9 binds to an LDLR, the receptor is destroyed along with the LDL particle, but this does not

DOI:10.2459/JCM.0000000000000360

happen if PCSK9 concentrations are intrinsically low. In this case the receptor can return to the surface of the cell and remove more cholesterol.[12] LDL-C levels lower than normal and subsequently reduced incidence of CHD have been observed in humans affected by PCSK9 mutations with loss of function.[13] On the contrary participants with PCSK9 mutations with gain of function have been shown to suffer from hypercholesterolemia with the development of premature CHD.[8] Moreover statins seem to increase PCSK9 levels, particularly in patients affected by familial hypercholesterolemia.[14] PCSK9 mutations, in fact, have been associated to autosomal dominant familial hypercholesterolemia, accounting for 1–2% of all familial hypercholesterolemia cases.[8,12] Early diagnosis of familial hypercholesterolemia is essential to reduce morbidity and mortality from premature atherosclerosis with an adequate lipid-lowering therapy.[15] The loss-of-function mutations of PCSK9 have been associated with a decrease of LDL-C levels. It has been estimated that the loss of one functional PCSK9 allele could prevent 88% of CVD events.[16] By inhibiting HMG-CoA = 3-hydroxy-3-methyl-glutaryl-CoA reductase, on the other site, statins increases the number of LDLR on the hepatic cell's surface and the level of PCSK9 increases to regulate the number of LDL-receptors, possibly contributing to a limitation on the effectiveness of statin in reducing LDL-C levels. Several clinical studies showed that administration of ezetimibe 10 mg/day is efficient in increasing the lipid-lowering effect of any statin by 15–26%, with the possibility to reach LDL-C threshold values recommended for an optimal control of cardiovascular risk.[17] This double inhibition, operated at the level of the enterocyte and hepatocyte, is up to date the best therapeutic option in the management of lipid disorders, preserving also the numerous pleiotropic effects of statins. PCSK9 inhibitors, unlike statins, may not have pleiotropic benefits on the unstable plaque but several studies indicate that PCSK9 promote inflammation, endothelial dysfunction, and hypertension with a mechanism independent of LDLR.[18] Additional research is needed to clarify the potential benefit of PCSK9 inhibition.

Clinical trials with proprotein convertase subtilisin kexin 9 inhibitors

Three pharmaceutical companies are producing monoclonal antibodies focused on PCSK9, which represents the most promising approach in reducing LDL-C levels, considering that a single injection can lower PCSK9 levels by up to 100% for more than 7 days. Amgen and Sanofi have conducted phase I and phase II clinical trials, and have demonstrated the safety and efficacy of the treatment confirming a 60–70% reduction of LDL-C with subcutaneous injections every 2 weeks. Possible side-effects consist of local reactions at injection sites. The possibility of serious side-effects from PCSK9 is conceivable because PCSK9 has been postulated to play role in different pathways as including neuronal apoptosis, regulation of sodium channels, pancreatic islet cell function and nervous system development.[19] However, humans affected by PCSK9 loss-of-function mutations or PCSK9-deficient mice do not appear to have any deficit, and it has been shown that people with LDL-C levels as low as 15 mg/dl are healthy.[20]

The PROFICIO program, the ODYSSEY program and the SPIRE program are ongoing studies with PCSK9 inhibitors. Patients at very high risk are being evaluated in the FOURIER trial, whereas patients hospitalized for acute coronary syndrome in the last 12 months with poor control of LDL-C levels are being evaluated in the ODYSSEY OUTCOMES trial.

Evolocumab

Evolocumab was tested in mice and monkey models demonstrating reductions of serum LDL-C and total cholesterol without adverse effects and no evidence of toxicity.[21,22] In phase I studies, moreover, AMG 145 reduced LDL-C levels by up to 66% in healthy and hypercholesterolemic statin-treated patients with minimal adverse effects.[23] The Program to Reduce LDL-C and Cardiovascular Outcomes Following Inhibition of PCSK9 in Different Populations (PROFICIO) consists of different phase II and III trials assessing LDL-C reduction and CVD outcomes. The LAPLACE-TIMI 57, a phase II trial, designed to assess the safety and efficacy of AMG 145 when added to statin therapy in patients with hypercholesterolemia, demonstrates the efficacy and safety of AMG145 in hypercholesterolemic patients treated with statins and has had a major role in determining dose selection and design of phase III cardiovascular outcome trials.[24] In the GAUSS trial, conducted in statin-intolerant patients, subcutaneous administration of monoclonal antibodies against PCSK9 reduced LDL-C levels with short-term tolerability.[25] The RUTHERFORD trial evaluated the safety and efficacy of AMG 145 in patients with heterozygous familial hypercholesterolemia (HeFH) with LDL-C of at least 2.6 mmol/l (100 mg/dl) despite statin therapy with or without ezetimibe. In this study patients were randomized 1:1:1 to AMG 145 350 mg, 420 mg or placebo-administered subcutaneously every 4 weeks with substantial reductions in LDL-C, up to 73%, in patients with HeFH with minimal adverse events and good tolerability.[26] The MENDEL trial shows that AMG145 administered in doses from 70 to 420 mg reduced LDL-C levels in every group of patients treated, by up to 48%. Moreover, no deaths or serious treatment-related adverse events were reported, assessing the use of AMG145 in long-term and larger studies.[27] The data from these four phase II trials were evaluated in the OSLER trial, a subsequent phase III study, which demonstrated that evolocumab administered every 4 weeks shows continued efficacy, safety and good tolerability over 1 year in

patients with hypercholesterolemia.[28] The GAUSSII trial evaluated evolocumab 140 mg administered every 2 weeks and 420 mg every 4 weeks in patients with hyperlipidemia with statin intolerance and showed a reduction of LDL-C levels of up to 53%, with a rate of adverse events, particularly muscle based, of 12%.[29] The LAPLACEII shows, in patients with hyperlipidemia treated with medium- or high-intensity statin therapy or with ezetimibe, a reduction of LDL-C levels of up to 75% by adding evolocumab 140 mg every 2 weeks or 420 mg in a monthly dose.[30] The DESCARTES trial shows the strong efficacy in reducing both PCSK9 levels (by up to 90%) and LDL-C levels (by up to 57%) in 1 week in patients with a baseline LDL-C of at least 75 mg/dl (≥1.9 mmol/l) undergoing treatment with evolocumab 420 mg monthly, with an adverse profile similar to placebo.[31] The MENDELII trial assessed monotherapy with evolocumab compared with ezetimibe or placebo in patients with hyperlipidemia, and showed a reduction of LDL-C levels up to 57%, with a rate of adverse events comparable to placebo.[32] The RUTHERFORDII trial assessed efficacy and safety of evolocumab 140 mg every 2 weeks or 420 mg every 4 weeks in patients with with a reduction of LDL-C levels up to 65% after 3 months of treatment.[33] Finally the TESLA-B trial assessed efficacy and safety of evolocumab in patients with homozygous familial hypercholesterolemia, showing a reduction of LDL-C levels up to 30% after 3 months of treatment. Phase II and phase III studies with evolocumab are reported in Table 1.[34]

Alirocumab

Several phase I and phase II studies have shown the efficacy and safety of alirocumab in lowering LDL-C levels, with rapid and substantial dose-dependent reduction and minimal adverse effects. Stein and colleagues[35] demonstrated that monoclonal antibody against PCSK9, REGN727/SAR236553 (REGN727), significantly reduces LDL-C levels in healthy volunteers and in patients with familial or nonfamilial hypercholesterolemia by up to 64%. McKenney and colleagues[36] observed that when added to atorvastatin, SAR236553 reduces LDL-C by 40–72%, and also in this case a dose and administration time-dependent effect was demonstrated. Interestingly a plateau effect for the higher doses was evidenced, suggesting that circulating and not intracellular PCSK9 is responsible for LDL-C regulation. Subsequently, Stein and colleagues[37] showed that REGN727, 150, 200 or 300 mg administered every 4 weeks, or 150 mg every 2 weeks, was well tolerated and led to further LDL-C reduction in patients with heterozygous familial hypercholesterolemia with elevated LDL-C levels despite statin therapy with or without ezetimibe. The most common adverse event was injection site reaction with only one drop-out patient. Roth and colleagues[38] conducted a randomized trial with patients affected by primary hypercholesterolemia and

Table 1 Clinical trial of evolocumab

Study	N[a] trial	Phase	Years	N[a] pts (of total)	Dosage (mg)	Baseline LDL-C	LDL reduction	Side-effects (vs. placebo)
LAPLACE-TIMI 57	NCT01380730	II	07-2011–03-2012	631	70-105-140 Q2W; 280-350-420 Q4W	≥85 mg/dl (≥2.2 mmol/)	−10%–>−66%	7 vs. 8%
GAUSS	NCT01375764	II	06-2011–05-2012	236	280-350-420 Q4W	193 mg/dl (4.99 mmol/l)	−34%–>−55%	Similar to placebo
RUTHERFORD	NCT01375751	II	07-2011–05-2012	205	350–420 QW4	≥100 mg/dl (≥2.6 mmol/l)	−44.1%–>−73%	Similar to placebo
MENDEL	NCT01375777	II	06-2011–03-2012	406	70-105-140 Q2W; 280-350-420 Q4W	100–190 mg/dl (2.6–4.99 mmol/l)	−39%–>−48%	50 vs. 46%[b]
YUKAWA	NCT01652703	II	07-2012–05-2013	310(422)	70-140 Q2W; 280-420 Q4W	>115 mg/dl (>3 mmol/l)	−63.7–−71%	51 vs. 38%[a]
OSLER	NCT01439880	III	10-2011–06-2012	1359	420 Q4W	139 mg/dl (3.6 mmol/l)	−53.1%	81.4 vs. 73.1%[c]
GAUSS-II	NCT01763905	III	01-2013–08-2013	427	140 Q2W; 420 Q4W	≥100 mg/dl (≥2.6 mmol/l)	−53%	<5%
LAPLACEII	NCT01763866	III	01-2013–08-2013	3593	140 Q2W; 420 Q4W	≥80 mg/dl (≥2.1 mmol/l)	−63–−75%	NA
DESCARTES	NCT01516879	III	01-2012–11-2013	800 (2120)	420 Q4W	≥75 mg/dl (≥1.9 mmol/l)	−46.7%–>56.7%	74.8 vs. 74.2%[a]
MENDEL2	NCT01763827	III	01-2013–10-2013	614 (614)	140 Q2W; 420 Q4W	100 ≤ LDL-C <190 mg/dl (2.6 mmol/l ≤ LDL-C <4.99 mmol/l)	−38%–>−57%	Similar to placebo
RUTHERFORD2	NCT01763918.	III	02-2013–12-2013	331 (415)	140 Q2W; 420 Q4W	≥100 mg/dl (≥2.6 mmol/l)	59.2%–>65.6%	Similar to placebo
TESLA-B	NCT01588496	III	04-2012–03-2014	49 (50)	420 Q4W	<426 mg/dl or >426 mg/dl (<11 mmol/l or ≥11 mmol/l)	−30.9%	36 vs. 63%

LDL-C, low-density lipoprotein cholesterol; NA, not applicable. [a]No deaths or serious treatment-related adverse events were reported. [b]Four patients taking evolocumab discontinued treatment because of an adverse event; no significant differences in adverse events rates based on dose or dose frequency; no serious or life-threatening adverse events. [c]Possibly related to evolocumab 5.6%. Q2W, every 2 weeks; Q4W, every 4 weeks

Table 2 Clinical trial of alirocumab

Study	N[a] trial	Phase	Years	N[a] pts (of total)	Dosage (mg)	Baseline LDL-C	LDL reduction	Side-effects
Stein *et al.*[35] – REGN727	NCT01026597	I	11-2009–10-2010	72(128)	50-100-150	≥100 mg/dl (≥2.6 mmol/l)	−38%–>−64.7%	Similar to placebo
	NCT01074372	I	03-2010–11-2010					
	NCT01161082	I	06-2010–05-2011					
McKenney *et al.*[36] – SAR236553/REGN727	NCT01288443	II	01-2011–12-2011	183	50-100-150 Q2W; 300 Q4W	≥100 mg/dl (≥2.6 mmol/l)	−40%–>−72%	0.54%[a]
Stein *et al.*[37] – REGN727/ SAR236553	NCT01266876	II	01-2011–11-2011	77	150 Q2W; 150-200-300 Q4W	≥100 mg/dl (≥2.6 mmol/l)	−28.9–−67.9%	Similar to placebo
Gaudet *et al.*[39] – SAR236553/ REGN727	NCT01266876 NCT01288469 NCT01288443	II	01-2011–11-2011	77	150 Q2W	≥100 mg/dl (≥2.6 mmol/l)	NA	1.5%
			01-2011–09-2011	99				
			01-2011–12-2011	183				
ODYSSEY-FHI	NCT01623115	III	07-2012–12-2014	486	75–150 Q2W	≥70 mg/dl (≥1.8 mmol/l)	−48.8%	NA
ODYSSEY-FHII	NCT01709500	III	12-2012–01-2015	249	75–150 Q2W	≥70 mg/dl (≥1.8 mmol/l)	−48.7%	NA
ODYSSEY-HIGHFH	NCT01617655	III	06-2012–01-2015	107	150 Q2W	≥160 mg/dl (≥4.13 mmol/l)	−46%	NA
ODYSSEY MONO	NCT01644474	III	07-2012–07-2013	103	75 Q2W	100–190 mg/dl (2.6–4.49 mmol/l)	−47%–>−54%	<2 vs. <4%
ODYSSEY COMBOI	NCT01644175	III	07-2012–04-2014	316	75–150 Q2W	≥70 mg/dl (≥1.8 mmol/l) with CVD or ≥100 mg/dl (≥2.6 mmol/l) with no CVD	−48% (maintain at 52 weeks)	NA
COMBOII	NCT01644188	III	08-2012–07-2015	720	75–150 Q2W	≥70 mg/dl (≥1.8 mmol/l) with CVD or ≥100 mg/dl (≥2.6 mmol/l) with no CVD	−50.6%	NA
ODYSSEY LONG TERM	NCT01507831	III	01-2012–11-2014	2341	150 Q2W	≥70 mg/dl (≥1.8 mmol/l)	−61%	3.85 vs. 2.83%[b]
ODYSSEY CHOICE I	NCT01926782	III	10-2013–05-2015	803	75 Q2W 300 Q4Ws	≥70 mg/dl (≥1.8 mmol/l) with CVD or ≥100 mg/dl (≥2.6 mmol/l) with no CVD	NA	NA
ODYSSEY CHOICE II	NCT02023879	III	12-2013–05-2016	233	75 Q2W 300 Q4W	≥70 mg/dl (≥1.8 mmol/l)with CVD or ≥100 mg/dl (≥2.6 mmol/l) with no CVD	NA	NA
ODYSSEY ALTERNATIVE	NCT01709513	III	09-2012–09-2016	314	75 Q2W (150 mg Q2W if LDL-C not at target after 8 weeks	≥70 mg/dl (≥1.8 mmol/l) with very high risk or ≥100 mg/dl (≥2.6 mmol/l) with high risk	−45%	NA
ODYSSEY OPTIONS I	NCT01730040	III	10-2012–05-2014	347		≥70 mg/dl (≥1.8 mmol/l) with CVD or ≥100 mg/dl (≥2.6 mmol/l) with no CVD	NA	NA
ODYSSEY OPTIONS II	NCT01730053	III	11-2012–05-2014	300		≥70 mg/dl (≥1.8 mmol/l) with or without LLT	NA	NA
ODYSSEY OUTCOME	NCT01663402	III	10-2012–01-2018	18 000		ACS patients	NA	NA
ODYSSEY OLE	NCT01954394	III	12-2013–06-2016	1200		HeFH	NA	NA

HeFH, heterozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; NA, not applicable [a] One case of leukocytoclastic vasculitis. [b] Mean percentage of adverse events considering injection site reactions (5.9 vs. 4.2%), myalgia (5.4 vs. 2.9%), neurocognitive events (1.2 vs. 0.5%) and ophthalmologic events (2.9 vs. 1.9%). Q2W, every 2 weeks; Q4W, every 4 weeks. LLT, lipid-lowering therapy; ACS, acute coronary syndrome.

showed that adding SAR236553 to either 10 mg of atorvastatin or 80 mg of atorvastatin led 90% of patients treated to achieve LDL-C levels less than 70 mg/dl (1.8 mmol/l), compared with only 17% of those under high-intensity statin therapy. Gaudet and associates,[39] moreover, demonstrated that alirocumab 150 administered twice per month resulted in a consistent reduction of lipoprotein(a), which could contribute in part to LDL-C lowering. The ODYSSEY program is a large phase III study with alirocumab and includes 14 global trials covering 23 500 participants, divided into three groups: HeFH, hypercholesterolemia in high-risk population and additional studies looking at monotherapy, statin intolerance and comparisons to atorvastatin or rosuvastatin with or without ezetimibe. The ODYSSEY FH I, II and HIGH FH trials assessed safety and efficacy of alirocumab 75 mg every 2 weeks in patients with HeFH and high risk or very high risk for CVD, showing a reduction of LDL-C up to 48% to week 24, with a similar rate of adverse events compared with placebo.[40] Alirocumab was demonstrated as superior to LDL-C-lowering therapies also in the ODYSSEY MONO, which compared this monoclonal antibody against PCSK9 versus ezetimibe, showing an LDL-C levels reduction of up to 54% at 24 week.[41] The ODYSSEY COMBO I and II trials evaluated safety and efficacy of alirocumab added to statin therapy as high as tolerated in hyperlipidemic high-risk patients compared with ezetimibe, resulting in an LDL-C level reduction of up to 50% at a dose of 75 mg twice a month. Similar rates of adverse events were also evidenced compared with ezetimibe.[42,43] The ODYSSEY LONG TERM trial evaluated the safety and tolerability of alirocumab in HeFH, high- and very high-risk patients (2341 under the highest tolerated statin therapy), showing LDL-C reduction of up to 61%, 80% of patients treated at target, similar adverse events compared with placebo but, moreover, a lower rate of cardiovascular events in alirocumab arm compared with placebo.[44] The ODYSSEY ALTERNATIVE evaluated the efficacy and safety of alirocumab compared with ezetimibe in very high-risk patients intolerant to statin with an LDL-C reduction of up to 45%, with 61% of patients at target and a lower rate of discontinuation of therapy compared with ezetimibe.[45] A similar trend has been observed in the COMBO II trial with maintenance of a 50% decrease in LDL-C at 52 weeks and similar data were confirmed also by the results from 78 weeks' follow-up of the ODYSSEY LONG TERM.[44] Further results are expected from the other ongoing trials ODYSSEY CHOICE I and II, ODYSSEY OPTIONS I and II, ODYSSEY OUTCOMES and ODYSSEY OLE.[22] Phase II and phase III studies with alirocumab are summarized in Table 2.

Bococizumab

In addition to the PROFICIO and ODYSSEY programs, other trials are evaluating the potential role of bococizumab (RN316) in Reducing the Occurrence of Major Cardiovascular Events in High Risk Subjects (SPIRE programs). SPIRE I and II (https://clinicaltrials.gov/ct2/show/NCT01975376, https://clinicaltrials.gov/ct2/show/NCT01975389) are evaluating the efficacy and safety of bococizumab, respectively in high-risk patients with baseline LDL-C between 100 mg/dl (2.6 mmol/l) and 70 mg/dl (1.8 mmol/l) and in high-risk patients with a baseline LDL-C not at target (>100 mg/d or >2.6 mmol/l). Other ongoing phase II trials are represented by the SPIRE-LDL (https://clinicaltrials.gov/ct2/show/NCT01968967) and the SPIRE HR (https://clinicaltrials.gov/ct2/show/NCT01968954), in patients with primary hyperlipidemia or mixed dyslipidemia at risk of cardiovascular events, and the SPIRE HF, in patients with heterozygous HeFH (https://clinicaltrials.gov/ct2/show/NCT01968980). The studies are ongoing and further results are expected.

Side-effects

Allergic reactions at the site of the injection are the most common side-effect as reported by many trials, with a similar event rate compared with placebo. The clinical trials with evolocumab reported no differences in the occurrence of side-effects compared with placebo.[25–35] Similar data are reported from ODYSSEY FH I, II and HIGH FH.[40] The ODYSSEY MONO reported a rate of side-effects similar to placebo.[41] The COMBO I and the ODYSSEY LONG TERM, on the contrary, assessed a risk of local site reaction inferior for alirocumab when compared with lipid-modifying therapies (respectively −5.3 vs. 2.8%, 5.9 vs. 4.2%).[42,44] Moreover site reactions were mild, transitory and did not cause study drug discontinuation. Finally the ODYSSEY ALTERNATIVE assessed a rate of study drug discontinuation because of adverse events lower with alirocumab compared with ezetimibe.[45] The OSLER trial reported similar rates of myalgia with alirocumab compared with lipid-modifying therapies (6.4 vs. 6.0%) but a higher rate was found by the ODYSSEY LONG TERM in the study group compared with placebo as reported (5.4 vs. 2.9%). A higher risk of developing neurocognitive disorders was reported by the OSLER trial (0.9 vs. 0.3%), a low percentage not differing from the one reported in the subgroup with very low LDL, but many data are controversial and a further and longer follow-up is needed.[46]

They seem in any case not to be related to very low LDL-C levels.[28] Regarding this concern, there have been reports of healthy participants with LDL-C levels as low as 15 mg/dl due to genetic mutation.[20] There are lots of concerns regarding the very low LDL-C levels (up to 0 mmol/l!) reached in these clinical trials. The COMBO I trial, for example, reported patients with LDL-C levels of 0.4 mmol/l, with no evidence of a major susceptibility to neurocognitive disorders or cardiovascular events at 18 months.[42] Discussion of potential harm

from very low LDL-C with possible development of cancer, hemorrhagic stroke and violent death will require further years of analysis and more evidence from outcomes trials to show a relationship with low LDL-C.[47] Moreover, conflicting data exist regarding the possible role of PCSK9 inhibitors on glucose homeostasis. In animal models, in fact, lack of PCSK9 led to cholesterol accumulation in pancreatic islet beta cells with subsequent impairment of insulin secretion, hyperglycemia and insulin insufficiency.[48] This suggestion has no confirmation in humans. In fact, in a large prospective cohort of patients it was recently demonstrated that the PCSK9 p.R46L LOF variant was not associated with impaired glucose homeostasis in humans.[49] These data are reassuring regarding the safety of PCSK9 inhibitors. PCSK9 is also expressed in other tissues in addition to the liver, with functions that are still not totally clarified. For example, PCSK9 is highly expressed in the intestine, particularly in the small bowel, where it seems to control the production of triglyceride lipoproteins and transintestinal cholesterol excretion.[50] Significant levels of PCSK9 were found also in kidneys where it seems that it could play a role in nephrogenesis.[51] PCSK9 located in smooth muscle cells regulates directly LDLR expression in macrophages with a paracrine action,[52] and it seems also to play a role in determining the content of atherosclerotic plaque.[53] Finally PCSK9 appears to play a role in neurogenesis and also in Alzheimer's disease by inducing deposition of beta-site amyloid precursor protein.[54]

Perspectives

Monoclonal antibodies against PCSK9 seem to be well tolerated, in terms of side-effects, although injections might not be particularly attractive for lifelong treatment. Despite this disadvantage, the efficacy of this new therapeutic approach is well documented and many patients, such as those suffering from HeFH, homozygous familial hypercholesterolemia or those unable to assume statin therapy owing to side-effects, may benefit from a therapy with a monoclonal antibody against PCSK9. Moreover, this approach will lead patients not on target to achieve stringent LDL-C target levels as recommended by the European guidelines, to new LDL levels never reached with the recent even most powerful lipid-lowering therapy.[55] However, the impact of PCSK9 inhibition in individuals with normal PCSK9 must be clarified.[56] Another question regarding these innovative medicines concerns the cost of therapy. In order to be cost-effective for patients, the PCSK9-inhibitors would need to cost around $2400 per year, strikingly lower than what manufacturers are currently charging for the two available drugs, according to a recent report.[57] Moreover the cost of therapy varies depending on whether it is a primary prevention (as in familial hypercholesterolemia) or secondary prevention (as in statin-intolerant patients). Actually estimates of annual price for these new medications are in a range of 6800–14 600 dollars, and given the great number of patients potentially eligible for the treatment with PCSK9 inhibitors, the potential overall cost could be huge.[58] However, further investigations are needed to clarify the risk of side-effects.

Conclusion

Reduction in LDL-C levels remains a mainstay of primary and secondary prevention in CVD. Many patients, including those suffering from HeFH, homozygous familial hypercholesterolemia or with consistent side-effects from statin may benefit from a lifelong therapy with monoclonal antibodies against PCSK9. Evolocumab, alirocumab and bococizumab seem to be well tolerated, except for an unattractive local site reaction. Many aspects on further side-effects of these treatments need clarification. Further years of analysis and more evidence from outcomes' trials are necessary to show a relationship between low LDL-C and medium-long-term side-effects.

Acknowledgements

There are no conflicts of interest.

References

1 Roth GA, Forouzanfar MH, Moran AE, *et al.* Demographic and epidemiologic drivers of global cardiovascular mortality. *N Engl J Med* 2015; **372**:1333–1341.
2 Ford ES, Ajani UA, Croft JB, *et al.* Explaining the decrease in U.S. deaths from coronary disease, 1980-2000. *N Engl J Med* 2007; **356**:2388–2398.
3 Stone NJ, Robinson JG, Lichtenstein AH, *et al.*, American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014; **129 (25 Suppl 2)**:S1–S45.
4 Cannon CP, Blazing MA, Giugliano RP, *et al.*, IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med* 2015; **372**:2387–2397.
5 Sniderman A, Thanassoulis G, Couture P, *et al.* Is lower and lower better and better? A re-evaluation of the evidence from the Cholesterol Treatment Trialists' Collaboration meta-analysis for low-density lipoprotein lowering. *J Clin Lipidol* 2012; **6**:303–309.
6 Gencer B, Lambert G, Mach F. PCSK9 inhibitors. *Swiss Med Wkly* 2015; **145**:w14094.
7 Seidah NG, Benjannet S, Wickham L, *et al.* The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A* 2003; **100**:928–933.
8 Abifadel M, Varret M, Rabès JP, *et al.* Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34**:154–156.
9 Wang YX, Wang YP, Zhang H, *et al.* Synthesis and biological evaluation of berberine analogues as novel up-regulators for both low-density-lipoprotein receptor and insulin receptor. *Bioorg Med Chem Lett* 2009; **19**:6004–6008.
10 Cariou B, Le May C, Costet P. Clinical aspects of PCSK9. *Atherosclerosis* 2011; **216**:258–265.
11 Zaid A, Roubtsova A, Essalmani R, *et al.* Proprotein convertase subtilisin/kexin type 9 (PCSK9): hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. *Hepatology* 2008; **48**:646–654.
12 Lambert G, Sjouke B, Choque B, Kastelein JJ, Hovingh GK. The PCSK9 decade. *J Lipid Res* 2012; **53**:2515–2524.
13 Cohen J, Pertsemlidis A, Kotowski IK, *et al.* Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat Genet* 2005; **37**:161–165.
14 Raal F, Panz V, Immelman A, Pilcher G. Elevated PCSK9 levels in untreated patients with heterozygous or homozygous familial hypercholesterolemia and the response to high-dose statin therapy. *J Am Heart Assoc* 2013; **2**:e000028.

15 Cuchel M, Bruckert E, Ginsberg HN, *et al.*, European Atherosclerosis Society Consensus Panel on Familial Hypercholesterolaemia. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J* 2014; **35**: 2146–2157.

16 Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006; **354**:1264–1272.

17 Califf RM, Lokhnygina Y, Cannon CP, *et al.* An update on the IMProved reduction of outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) design. *Am Heart J* 2010; **159**:705–709.

18 Urban D, Pöss J, Böhm M, Laufs U. Targeting the proprotein convertase subtilisin/kexin type 9 for the treatment of dyslipidemia and atherosclerosis. *J Am Coll Cardiol* 2013; **62**:1401–1408.

19 Maxwell KN, Breslow JL. Antibodies to PCSK9: a superior way to lower LDL cholesterol? *Circ Res* 2012; **111**:274–277.

20 Lepor NE, Contreras L, Desai C, Kereiakes DJ. The potential role of anti-PCSK9 monoclonal antibodies in the management of hypercholesterolemia. *Rev Cardiovasc Med* 2014; **15**:290–307.

21 Chan JC, Piper DE, Cao Q, *et al.* A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. *Proc Natl Acad Sci U S A* 2009; **106**:9820–9825.

22 Ni YG, Di Marco S, Condra JH, *et al.* A PCSK9-binding antibody that structurally mimics the EGF(A) domain of LDL-receptor reduces LDL cholesterol in vivo. *J Lipid Res* 2011; **52**:78–86.

23 Dias CS, Shaywitz AJ, Wasserman SM, *et al.* Effects of AMG 145 on low-density lipoprotein cholesterol levels: results from 2 randomized, double-blind, placebo-controlled, ascending-dose phase 1 studies in healthy volunteers and hypercholesterolemic subjects on statins. *J Am Coll Cardiol* 2012; **60**:1888–1898.

24 Kohli P, Desai NR, Giugliano RP, *et al.* Design and rationale of the LAPLACE-TIMI 57 trial: a phase II, double-blind, placebo-controlled study of the efficacy and tolerability of a monoclonal antibody inhibitor of PCSK9 in subjects with hypercholesterolemia on background statin therapy. *Clin Cardiol* 2012; **35**:385–391.

25 Sullivan D, Olsson AG, Scott R, *et al.* Effect of a monoclonal antibody to PCSK9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: the GAUSS randomized trial. *JAMA* 2012; **308**:2497–2506.

26 Raal F, Scott R, Somaratne R, *et al.* Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation* 2012; **126**:2408–2417.

27 Koren MJ, Scott R, Kim JB, *et al.* Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study. *Lancet* 2012; **380**:1995–2006.

28 Koren MJ, Giugliano RP, Raal FJ, *et al.*, OSLER Investigators. Efficacy and safety of longer-term administration of evolocumab (AMG 145) in patients with hypercholesterolemia: 52-week results from the Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) randomized trial. *Circulation* 2014; **129**:234–243.

29 Stroes E, Colquhoun D, Sullivan D, *et al.*, GAUSS-2 Investigators. Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the GAUSS-2 randomized, placebo-controlled phase 3 clinical trial of evolocumab. *J Am Coll Cardiol* 2014; **63**:2541–2548.

30 Robinson JG, Rogers WJ, Nedergaard BS, *et al.* Rationale and design of LAPLACE-2: a phase 3, randomized, double-blind, placebo- and ezetimibe-controlled trial evaluating the efficacy and safety of evolocumab in subjects with hypercholesterolemia on background statin therapy. *Clin Cardiol* 2014; **37**:195–203.

31 Blom DJ, Hala T, Bolognese M, *et al.*, DESCARTES Investigators. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. *N Engl J Med* 2014; **370**:1809–1819.

32 Koren MJ, Lundqvist P, Bolognese M, *et al.*, MENDEL-2 Investigators. Anti-PCSK9 monotherapy for hypercholesterolemia: the MENDEL-2 randomized, controlled phase III clinical trial of evolocumab. *J Am Coll Cardiol* 2014; **63**:2531–2540.

33 Raal FJ, Stein EA, Dufour R, *et al.*, RUTHERFORD-2 Investigators. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; **385**:331–340.

34 Raal FJ, Honarpour N, Blom DJ, *et al.*, TESLA Investigators. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; **385**:341–350.

35 Stein EA, Mellis S, Yancopoulos GD, *et al.* Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med* 2012; **366**:1108–1118.

36 McKenney JM, Koren MJ, Kereiakes DJ, *et al.* Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. *J Am Coll Cardiol* 2012; **59**:2344–2353.

37 Stein EA, Gipe D, Bergeron J, *et al.* Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial. *Lancet* 2012; **380**:29–36.

38 Roth EM, McKenney JM, Hanotin C, *et al.* Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. *N Engl J Med* 2012; **367**:1891–1900.

39 Gaudet D, Kereiakes DJ, McKenney JM, *et al.* Effect of alirocumab, a monoclonal proprotein convertase subtilisin/kexin 9 antibody, on lipoprotein(a) concentrations (a pooled analysis of 150 mg every two weeks dosing from phase 2 trials). *Am J Cardiol* 2014; **114**:711–715.

40 Kastelein JJ, Robinson JG, Farnier M, *et al.* Efficacy and safety of alirocumab in patients with heterozygous familial hypercholesterolemia not adequately controlled with current lipid-lowering therapy: design and rationale of the ODYSSEY FH studies. *Cardiovasc Drugs Ther* 2014; **28**:281–289.

41 Roth EM, McKenney JM. ODYSSEY MONO: effect of alirocumab 75 mg subcutaneously every 2 weeks as monotherapy versus ezetimibe over 24 weeks. *Future Cardiol* 2015; **11**:27–37.

42 Kereiakes DJ, Robinson JG, Cannon CP, *et al.* Efficacy and safety of the proprotein convertase subtilisin/kexin type 9 inhibitor alirocumab among high cardiovascular risk patients on maximally tolerated statin therapy: the ODYSSEY COMBO I study. *Am Heart J* 2015; **169**:906.e13–915.e13.

43 Cannon CP, Cariou B, Blom D, *et al.*, ODYSSEY COMBO II Investigators. Efficacy and safety of alirocumab in high cardiovascular risk patients with inadequately controlled hypercholesterolaemia on maximally tolerated doses of statins: the ODYSSEY COMBO II randomized controlled trial. *Eur Heart J* 2015; **36**:1186–1194.

44 Robinson JG, Farnier M, Krempf M, *et al.*, ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015; **372**:1489–1499.

45 Moriarty PM, Jacobson TA, Bruckert E, *et al.* Efficacy and safety of alirocumab, a monoclonal antibody to PCSK9, in statin-intolerant patients: design and rationale of ODYSSEY ALTERNATIVE, a randomized phase 3 trial. *J Clin Lipidol* 2014; **8**:554–561.

46 Sabatine MS, Giugliano RP, Wiviott SD, *et al.*, Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015; **372**:1500–1509.

47 Stein EA, Raal FJ. Targeting LDL: is lower better and is it safe? *Best Pract Res Clin Endocrinol Metab* 2014; **28**:309–324.

48 Mbikay M, Sirois F, Gyamera-Acheampong C, *et al.* Variable effects of gender and Western diet on lipid and glucose homeostasis in aged PCSK9-deficient C57BL/6 mice CSK9PC57BL/6. *J Diabetes* 2015; **7**:74–84.

49 Bonnefond A, Yengo L, Le May C, *et al.*, DESIR study group. The loss-of-function PCSK9 p.R46L genetic variant does not alter glucose homeostasis. *Diabetologia* 2015; **58**:2051–2055.

50 Cariou B, Si-Tayeb K, Le May C. Role of PCSK9 beyond liver involvement. *Curr Opin Lipidol* 2015; **26**:155–161.

51 Seidah NG, Benjannet S, Wickham L, *et al.* The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci USA* 2003; **100**:928–933.

52 Ferri N, Tibolla G, Pirillo A, *et al.* Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by culture smooth muscle cells reduces macrophages LDLR levels. *Atherosclerosis* 2012; **220**:381–386.

53 Kuhnast S, Van der Hoorn JW, Pieterman EJ, *et al.* Alirocumab inhibits atherosclerosis, improves the plaque morphology, and enhances the effect of a statin. *J Lipid Res* 2014; **55**:2103–2112.

54 Jonas MC, Costantini C, Puglielli L. PCSK9 is required for the disposal of nonacetylated intermediates of the nascent membrane protein BACE1. *EMBO Rep* 2008; **9**:916–922.
55 Jensen MD, Ryan DH, Apovian CM, *et al*., American College of Cardiology/American Heart Association Task Force on Practice Guidelines; Obesity Society. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation* 2014; **129 (25 (Suppl 2))**:S102–S138.
56 Lambert G, Petrides F, Chatelais M, *et al.* Elevated plasma PCSK9 level is equally detrimental for patients with nonfamilial hypercholesterolemia and heterozygous familial hypercholesterolemia, irrespective of low-density lipoprotein receptor defects. *J Am Coll Cardiol* 2014; **63**:2365–2373.
57 Institute for Clinical and Economic Review. PCSK9 inhibitors for treatment of high cholesterol: effectiveness, value, and value-based price benchmarks draft report. Published September 8, 2015.
58 O'Riordan M. PCSK9 inhibitors not cost-effective at current Price: ICER Review. Heartwire from Medscape. http://www.medscape.com/viewarticle/850715,09.09.2015.